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Intra-individual variation in urinary iodine concentrations: effect of adjustment on population distribution using two and three repeated spot urine collections

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Contribution of each author:

Karen E Charlton: Conceptualisation of study design, drafting initial manuscript

Marijka J Batterham: Statistical analysis, editing of manuscript.

Li Min Tan Buchanan: Participant recruitment, data collection, data entry, editing of manuscript.

Dorothy Mackerras: Statistical interpretation, editing of manuscript.

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Summary

Article focus

- The iodine status of populations is defined by calculating median urinary iodine concentrations (UIC) from spot urine samples collected in surveys.
- High intra-individual (day-to-day) variation in urinary iodine excretion leads to an overdispersed distribution.
- Methods used in other areas of research to correct for intra-individual variation were applied to three repeated spot urine collections from a sample of older Australians to estimate a UIC distribution more reflective of long-term usual status.

Key messages

- Collection of a single urine sample leads to incorrect conclusions about the extremes of the population distribution of UIC.
- Adjustment using analysis of variance reduced the spread of the distribution more than calculating an average of each person's samples. Application of this method to surveillance of population iodine status and iodine fortification programmes could permit a more detailed assessment of the population and relevant subgroups.
- In this sample of older adults, three spot urine collections did not add value compared to two collections.

Strengths and limitations:

Older adults, who typically have a less varied diet than younger populations, were sampled therefore generalizability to other age groups may be limited.

Abstract

Background: Iodine deficiency is assessed on a population level by comparing median spot urinary iodine concentrations (UIC) against references for pregnant and non-pregnant states. Intra-individual variation of a single UIC results in an overdispersed distribution and consequent errors in estimating the prevalence of deficiency and excess.

Methods: UIC data collected from 84 healthy volunteers, 60-95y from New South Wales, Australia, prior to the mandatory fortification programme, was used to determine the effect of adjustment for intra-individual variation on estimations of iodine deficiency and the population distribution. Three spot urine samples were collected, each one week apart. Repeated measures analysis of variance determined between-person (s_b) and total (s_{obs}) standard deviations. Adjusted UIC values were calculated as [(person's UIC – group mean) x (s_b/s_{obs})] + group mean, and a corrected UIC distribution calculated.

Results: The s_b/s_{obs} for using 3-samples and 2-samples was 0.83 and 0.79, respectively. Following adjustment for intra-individual variation, the proportion with UIC < 50 ug/L reduced from 33 % to 19%, while the proportion with UIC ≥ 100 ug/L changed from 21% to 17%. The 95th centile UIC decreased from 176 to 136 μg/L. Adjustment by taking averages yielded a lesser degree of contraction in the distribution than the analysis of variance method.

Conclusions: The addition of information about intra-individual variability has potential for increasing the interpretability of UIC data collected to monitor the iodine status of a population.

Keywords: iodine, urinary iodine concentration, intra-individual variation, distribution

Introduction

Iodine deficiency is one of the most common nutrient deficiencies in the world, with almost one billion people affected. Populations that consume diets that contain small amounts of fish and seafood, moderate to low quantities of milk and dairy products, and include locally produced fruits and vegetables grown in iodine-poor soils are likely to be iodine deficient. Iodine deficiency impacts across the life stages.[1] It affects growth and development (both cognitive and motor) during pregnancy, infancy and childhood. In older adults, iodine deficiency may play a role in declining cognitive function.[1] Consequently, good quality population-level data on iodine status is required to assess population status and design strategies which correct any deficiency but avoid introducing excessive intakes. The iodine status of populations is defined by calculating urinary iodine concentrations (UIC) from spot urine samples collected in a representative sample and comparing the median UIC (MUIC) against reference ranges.[2] Daily urinary excretion of iodine closely reflects iodine intake in non-pregnant populations therefore MUIC of a group is considered to be a valid biomarker of the status of that group.[3] However, the concentration measured in a single spot sample has large variation from day-to-day within individuals,[4-6]. This increases the spread of the distribution [7, 8] so that it does not reflect the range of long-term or 'usual' iodine status around the median in the population. It is the usual intake of iodine, not the intake on any one day that determines iodine status of groups. Consequently, a method to reduce or remove the effects of the measurement error due to the intra-individual variation that results from collecting a single spot urine sample from each survey participant would allow greater description of the population status.

Several methods exist to correct for intra-individual variation in population survey data. One method is to collect multiple days of data on each survey participant and average the data for each participant. This has substantial logistical costs when conducting a national survey.

Another method is to apply a correction factor to the distribution.[8, 9] This requires estimating the correction factor, for example by collecting multiple samples from a representative subset of the survey population

This second method has been applied many times to dietary intake data [10-12] but has been applied less frequently to biochemical data.[13-15] In a survey of indigenous Australian adolescents with MUIC of 51 ug/L, correcting the distribution based on two urine samples per person, reduced the 95th centile from 129 ug/L to 92 ug/L.[13, 14] have highlighted the widespread misuse of calculating the proportions of UIC below the cut-off level of 100 ug/L to estimate the prevalence of iodine deficiency. This will over- or underestimate the proportion with deficiency depending on the location of the median. In the previous example,[13] the raw data would be misinterpreted as showing that 90% were <100 ug/L, whereas this was 97% after correction for within person variation. In clearly deficient populations, this difference is not important for program planning. However as a population approaches sufficiency, accurate estimation becomes more important for refining programs. Similarly in replete populations, an accurate estimate of the high intakes is needed to assess whether part of the population is reaching potentially adverse levels.

Remote indigenous people have a different lifestyle from that of urbanised non-Indigenous Australians. In this study, we investigate the intra-individual variation in UIC in older non-Indigenous Australians, living in an urban iodine deficient area prior to the introduction of mandatory fortification. We extend previous work by examining the impact of having two versus three samples for calculating the correction factor and compare this to the effect of averaging the results for each person.

Methods

Between May and September 2009, 110 English-speaking men and women aged 60 - 95 years volunteered for a study that investigated the association between iodine status and cognition. Volunteers were recruited from a random selection of aged care facilities (independent, assisted and low care living) in the Illawarra region, south of Sydney in Australia. Of the 110 participants, 84 subjects (25 men) met the study inclusion criteria and were enrolled. Twenty-six subjects were excluded due to: a) diagnosed dementia and/or Alzheimer's disease, b) cognitive decline as indicated by a Mini-Mental State Examination (MMSE[9] score of <=23,[16] c) a previous stroke, d) current use of thyroxine or any other medications that may affect memory, 5) uncontrolled hypertension (blood pressure (BP) ≥ 160/95 mm Hg), and e) uncontrolled diabetes (blood glucose (BG) ≥ 7.8 mmol/l). The study protocol was approved by the Human Research Ethics Committee of the University of Wollongong and all participants provided written informed consent.

Weight (Tanita Scale, TBG622, Tanita Inc., Tokyo, Japan) and height (stadiometer) of subjects were measured. Body mass index was calculated as the ratio of weight (kg) divided by height squared (m^2).

Participants were provided with written instructions for spot urine sample collections, which included collection of the first voiding of the day, on the same day each week, where possible, over a three-week period. Urine samples were stored at -80°C and batch-analysed by the accredited laboratory of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital (Sydney, NSW, Australia). UIC was analysed using an adaptation of the Sandell-Kolthoff method using a ammonium persulphate digestion and microplate reading.[17] The coefficient of variation (CV) of the urinary iodine assay in the

ICPMR laboratory is 16.7% at 46 ± 7.72 µg/L, 5.8% at 153 ± 8.9 µg/L, and 8.65% at 347 ± 30 µg/L. The group MUIC was compared to population-specific reference values.[2]

The UIC data were transformed using the natural logarithm to improve normality. Repeated measures analysis of variance was performed to determine the between-person (s_b) and total (s_{obs}) standard deviations. An adjusted log UIC value was calculated for each person as [18]: Adjusted UIC = [(person's day 1 UIC – group mean for day 1) * ($s_b \div s_{obs}$)] + group mean for day 1. (Equation 1)

The results were exponentiated. The adjustment procedure was performed twice using SAS (V9.2, SAS Cary, NC). First the correction factor (s_b/s_{obs}) was calculated using all three replicates, then it was calculated using only the first two replicates.

We also calculated the average for each person using all three replicates and for the first two replicates. Centiles of the distribution and the proportion below selected values were calculated for the raw Day 1 data and for distributions derived using adjustment or averaging. Descriptive analyses were performed using IBM SPSS (V19.0 IBM Corporation, Armonk NY).

Results

Sociodemographic characteristics of the study participants are shown in Table 1.

Table 1
Demographic and clinical characteristics of the study subjects (n = 84)

Characteristics	Subjects (*n* =84) (%)
Sex (%)	
Men	25(30%)
Women	59(70%)
Age (years)	74 ± 8[a]
BMI[b]	
Mean	28.4 ± 4.7[a]
Underweight (< 18.5 kg/m^2)	0 (0%)
Normal Weight (18.5 - 24.9 kg/m^2)	16(19%)
Overweight (≥ 25 kg/m^2)	45 (54%)
Obese ≥ (30 kg/m^2)	23 (27%)
MNA score	
Mean	27.2 ± 3.6 [a]
Malnourished (< 17 points)	0 (0%)
At risk of Malnutrition (17 - 23.5 points)	4 (5%)
Well-nourished (≥ 24 points)	78 (93%)
Unknown	2 (2%)
Barthel Index[17]	
Able to independently perform activities of daily living (>50 points)	84 (100%)
MMSE score[18]	
0 to 23	0 (0%)
24-30	83 (99%)
Unknown	1 (1%)
Education level	
≤ Year 12	39 (47%)
> Year 12	44 (52%)
Unknown	1 (1%)

[a] Mean ± standard deviation
[b] Calculated as kg/m^2

MUIC of the study population using the first spot urine collection indicated mild iodine deficiency (65.5 (IQR 42; 89)) µg/L). Correlations for transformed urinary iodine concentration values were: Days 1 and 2: r=0.48; p<0.01; Days 1 and 3: r=0.43; p<0.01; Days 2 and 3: r=0.41; p<0.01. The distribution of urinary iodine concentrations calculated by the different methods is shown in Table 2 and Figure 1. The s_b/s_{obs} was 0.83 when calculated using the three replicates and 0.79 when calculated using two replicates; i.e. the contraction in the distribution was slightly less with two replicates than three. Compared to the raw distribution, adjustment and averaging both reduced the spread of the distribution, especially

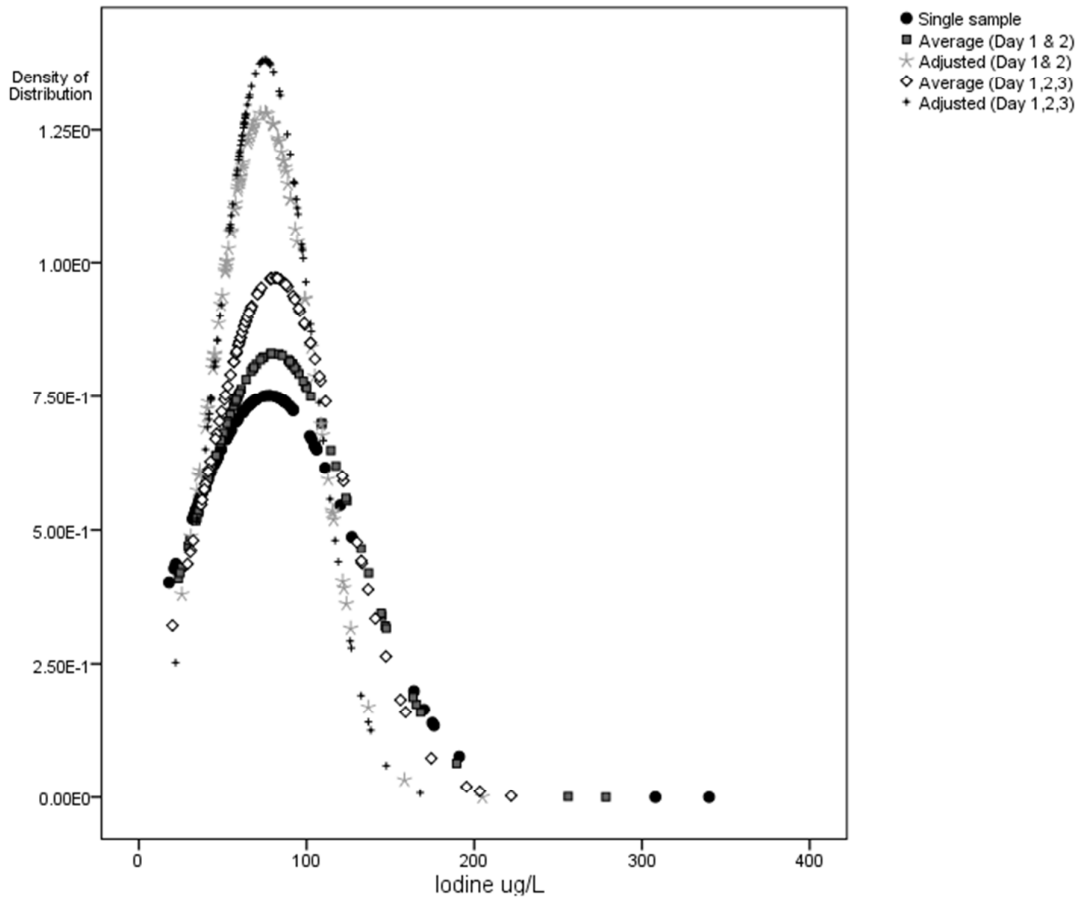
at the upper end owing to the right skew in the data. Following adjustment using the three urine collections, the MUIC remained unchanged but the IQR was shifted upwards 65.2 (55; 94) while the upper end of the distribution (95th centile) changed from 176 to 136 µg/L. The percentage of participants with UIC ≥ 100 ug/L decreased from 21 % to 17 % following adjustment.

Table 2
Urinary iodine concentration distribution, raw data from one day, after adjustment for intra-individual variation of two and three spot sample collections, and averages of multiple collections

	Urinary iodine concentration distributions (ug/L)				
Centile	Raw data for Day 1	Average of Day 1 and Day 2	Day 1 corrected using 2 replicates	Average of Days 1, 2 and 3	Day 1 corrected using 3 replicates
5th	32.25	29.7	35.9	31.3	40.0
10th	35.50	35.5	40.8	38.3	43.0
25th	42.25	48.6	52.1	55.2	55.4
50th	65.5	65.5	66.8	69.0	65.2
75th	89.5	99.1	90.0	98.9	94.3
90th	123.5	146	115.8	138.8	118.0
95th	175.8	167.4	125.7	170.5	135.8
Maximum	340.0	278.5	204.8	222.0	167.9
Percent < 20ug/L	1%	0%	0%	0%	0%
Percent <50 ug/L	33%	27%	21%	20%	19%
Percent ≥ 100 ug/L	21%	24%	18%	24%	17%

Taking an average of the three replicates also yielded similar results to taking an average of only two replicates. The averaging method contracted the distribution less than the adjustment method and, in particular had less effect in drawing the upper tail towards the median.

Figure 1 Population distribution of urinary iodine, according to number of days of spot urine collection



Discussion

In this population with a suboptimal iodine status, the collection of a single urine sample from each participant would lead to different conclusions about the extremes of the population distribution of UIC. This has also been shown previously in Indigenous Australian adolescents [13] and young Swiss women.[14]

In our population, there was no gain in having three, rather than two, samples as both adjustment factors were approximately 0.8 on the natural logarithmic scale. This means that the standard deviation of the final distribution is 80% of the original. The lower the ratio, the higher the correction - for example, a ratio of 0.5 would have resulted in a distribution with a standard deviation of half the width of the original.[19] We do not know whether having a greater number of replicates, such as seven or 14, or including different seasons, would have yielded the same result. Our urinary findings are consistent with the low variability in dietary iodine intake assessed at the same time in this population living in low level residential aged care facilities.[20] A similar adjustment ratio of 0.69 on the natural logarithmic scale for UIC has been reported in indigenous adolescents from the Darwin area, whose dietary patterns are limited in variety.[13] We hypothesize that the degree of adjustment would be larger in other populations that have more variety in food intake.

Heterogeneity in the iodine content of different foods and their frequency of consumption in different populations will affect the magnitude of intra-individual variability in UIC. This suggests that the intra-individual variability in UIC would change after a fortification program is introduced. It could be further hypothesised that the intra-individual variability might increase if one or a small number of foods is fortified, particularly at high concentrations. In Tasmania, the interquartile range widened as the MUIC value increased following iodisation of salt (25-65g iodine/kg salt) used in bread. Pre-fortification, MUIC in schoolchildren was 73 (IQR 56-100) µg/L, which increased to 108 (73-158) µg/L following voluntary fortification, and to 132µg/L (96-198) µg/L) post mandatory fortification. [21] However, it is not possible to compare these results to studies which report other parameters for the UIC distribution such as the range [14]or the 10-90th centiles.[22] It is less clear whether variability would increase or decrease if a wide range of foods are fortified and/or at

a lower concentration. We hypothesise that adjustment factors need to be reassessed if iodine intake changes and multiple factors might be needed if there is geographical variation in iodine status within a country.

The greater narrowing in population distribution that results from the adjustment method, compared to the averaging method, is expected if multiple days are needed to estimate the long-term UIC for an individual. This is because the average of few days of urinary collection would still contain intra-individual variation. However, studies that collect a greater number of replicate samples than in the current study are still needed to compare the distribution determined by statistical adjustment to a directly assessed long-term average UIC.

Our study sample comprised older adults, a group who often have impaired renal function. We have previously suggested that spot urinary iodine concentrations may be under-estimating 24 hr excretion in this study population [20] but this would not impact on intra-individual variability of UIC which is the topic of the current paper. Any variation in day-to-day fluid intake would be included in the changing UIC for each person in the study on each day of collection. However, as the samples were all collected within a three week period, we would not have included any variation in fluid intake related to seasons. This would have underestimated the degree of adjustment in this population. However a reduction in total fluid intake, and therefore urine volume, might or might not affect the day-to-day variation.

In our study, we had replicate samples on all participants. A more logistically feasible alternative in a large survey is to collect the replicates in a representative sub-set and apply the adjustment factor calculated in the sub-set to the whole population. It may be necessary to subdivide the population, for example, by age and sex, and ensure that there are enough participants in each sub-division to permit a suitable range of adjustment factors to be

calculated. The method we have used [18] is the simplest of several methods that have a similar purpose.[23] The disadvantage of collecting replicates in a sub-set only is that, although an estimate of the usual UIC distribution of the population is obtained, the method treats each individual as representative of a larger group and so the value calculated for each individual in Equation 1 is theoretical. Therefore, if it is desired to link intake with excretion at an individual level, then replicate information about both intake and excretion for each survey participant would be preferable but it is also possible to correct a regression coefficient for within person variability.[24] In addition, the method we used assumes that the data can be normalised. If this is not true, then alternatives include calculating an average for each participant [25] or using a complex method such as the National Cancer Institute method.[26]

Conclusion

In a sample of healthy older Australian adults who were iodine deplete, the use of two or three spot urine samples for adjustment of intra-individual variation in urinary iodine concentration resulted in a narrowed population distribution, particularly at the upper end. Statistical adjustment yielded a stronger correction than averaging the replicates. There was no important gain in collecting a third sample in this population; however this finding might be specific to our group and not generalizable to other age groups. The impact of the adjustment in narrowing the distribution would be greater in groups with more varied dietary intakes, and therefore wider intra-individual variation in UIC. These results provide a case for further work to investigate the usefulness of determining adjustment factors to remove intra-individual variability as part of population assessment of iodine status.

References

1 Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. *Lancet* 2008;**372**:1251-62.

2 World Health Organization. Assessment of iodine deficiency disorders and monitoring their elimination. Geneva: WHO 2007.

3 Ristic-Medic D, Piskackova Z, Hooper L*, et al.* Methods of assessment of iodine status in humans: a systematic review. *Am J Clin Nutr* 2009;**89**:2052S-69S.

4 Rasmussen LB, Ovesen L, Bulow I*, et al.* Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. *Br J Nutr* 2002;**87**:61-9.

5 Rasmussen LB, Ovesen L, Christiansen E. Day-to-day and within-day variation in urinary iodine excretion. *Eur J Clin Nutr* 1999;**53**:401-7.

6 Als C, Helbling A, Peter K*, et al.* Urinary iodine concentration follows a circadian rhythm: A study with 3023 spot urine samples in adults and children. *J Clin Endocrinol Metab* 2000;**85**:1367-9.

7 Andersen S, Karmisholt J, Pedersen KM*, et al.* Reliability of studies of iodine intake and recommendations for number of samples in groups and in individuals. *Br J Nutr* 2008;**99**:813-8.

8 Armstrong BK, White E, Saracci R. *Principles of exposure measurement in epidemiology*. Oxford; New York: Oxford University Press 1992.

9 Dyer AR, Shipley M, Elliott P. Urinary electrolyte excretion in 24 hours and blood-pressure in the Intersalt study. 1. Estimates of reliability *Am J Epi* 1994;**139**:927-39.

10 Subar AF, Dodd KW, Guenther PM*, et al.* The Food Propensity Questionnaire: Concept, development, and validation for use as a covariate in a model to estimate usual food intake. *J Am Diet Assoc* 2006;**106**:1556-63.

11 Freedman LS, Midthune D, Carroll RJ, *et al.* Adjustments to improve the estimation of usual dietary intake distributions in the population. *J Nutr* 2004;**134**:1836-43.

12 Kipnis V, Midthune D, Buckman DW, *et al.* Modeling Data with Excess Zeros and Measurement Error: Application to Evaluating Relationships between Episodically Consumed Foods and Health Outcomes. *Biometrics* 2009;**65**:1003-10.

13 Mackerras DEM, Singh GR, Eastman CJ. Iodine status of Aboriginal teenagers in the Darwin region before mandatory iodine fortification of bread. *Med J Aust* 2011;**194**:126-30.

14 Zimmermann MB, Andersson M. Assessment of iodine nutrition in populations: past, present, and future. *Nutr Rev* 2012;**70**:553-70.

15 Looker AC, Dallman PR, Carroll MD, *et al.* Prevalence of iron deficiency in the United States. *JAMA* 1997;**277**:973-6.

16 Folstein MF, Folstein SE, McHugh PR. Mini-Mental State - practical method for grading cognitive state of patients for clinician *J Psych Res* 1975;**12**:189-98.

17 Ohashi T, Yamaki M, Pandav CS, *et al.* Simple microplate method for determination of urinary iodine. *Clin Chem* 2000;**46**:529-36.

18 Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Reference SCotSEoD, Intakes. *Dietary Reference Intakes: Applications in Dietary Planning*. Washington, DC: The National Academies Press 2003.

19 Baines J, Cunningham J, Leemhuis C, *et al.* Risk Assessment to Underpin Food Regulatory Decisions: An Example of Public Health Nutritional Epidemiology. *Nutrients* 2011;**3**:164-85.

20 Tan L, Charlton KE, Roodenrys S, *et al.* Validation of an iodine-specific Food Frequency Questionnaire to estimate dietary iodine intake in older Australians. *Nutr Diet* 2012;**70**:71-8.

21 Depaoli K, Seal JA, Taylor R. Progressive improvements in iodine status in Tasmania with voluntary then mandatory foritifcation: Preliminary results from the 2011 Tasmanian Urinary Iodine Survey. *Nutr Diet* 2012;**69 (Suppl 1)**:53.

22 U.S. Centers for Disease Control and Prevention. *Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population 2012*. Atlanta (GA): National Center for Environmental Health 2012.

23 National Cancer Institute. Usual Dietary Intakes: SAS Macros for Analysis of a Single Dietary Component. 2010: http://riskfactor.cancer.gov/diet/usualintakes/macros_single.html.

24 Sempos CT, Johnson NE, Smith EL*, et al.* Effects of intraindividual and interindividual variation in repeated dietary records *Am J Epi* 1985;**121**:120-30.

25 Food Standards Australia New Zealand (FSANZ). *The 2007 Australian National Children's Nutrition and Physical Activity Survey Volume Eight: Dietary exposure to food sourced chemicals*. Canberra: Department of Health and Ageing 2012.

26 Tooze JA, Kipnis V, Buckman DW*, et al.* A mixed-effects model approach for estimating the distribution of usual intake of nutrients: The NCI method. *Stat Med* 2010;**29**:2857-68.

This paper is not a reliability study but we have used the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) checklist as this seems to be the closest in terms of study design.

		Page in manuscript where information appears
TITLE AND ABSTRACT		
1. Identify in title or abstract that interrater/intrarater reliability or agreement was investigated.	Title reflects the analysis performed" **Intra-individual variation in urinary iodine concentrations: Effect of adjustment on population distribution using two and three repeated spot urine collections .** "Abstract states "UIC data collected from 84 healthy volunteers, 60-95y from New South Wales, Australia, prior to the mandatory fortification programme was used to determine the effect of adjustment for intra-individual variation on estimations of iodine deficiency and the population distribution."	Page 1 and 2
INTRODUCTION		
2. Name and describe the diagnostic or measurement device of interest explicitly	Measurement is repeated spot urine samples for analysis of Urinary Iodine Concentration (UIC). This is explained in detail, and the methodological problems associated with the single spot urine (currently the recommended method for population-level assessment of iodine status).	Pages 4 (2nd para),5, and 6
3. Specify the subject population of interest.	This is adequately described as follows: *Remote indigenous people have a different lifestyle from that of urbanised non-Indigenous Australians. In this study, we investigate the intra-individual variation in UIC in older non-Indigenous Australians, living in an urban iodine deficient area prior to the introduction of mandatory fortification. We extend previous work by examining the impact of having two versus three samples for calculating the correction factor and compare this to the effect of averaging the results for each person.* *Between May and September 2009, 110 English-speaking men and women aged 60 - 95 years volunteered for a study that investigated the association between iodine status and cognition. Volunteers were recruited from a random selection of aged care facilities (independent, assisted and low care living) in the Illawarra region, south of Sydney in Australia. Of the 110 participants, 84 subjects (25 men) met the study inclusion criteria and were enrolled. Twenty-six subjects were excluded due to: a) diagnosed dementia and/or Alzheimer's disease, b) cognitive decline as indicated by a Mini-Mental State Examination (MMSE score of <=23,[16] c) a previous stroke, d) current use of thyroxine or any other medications that may affect memory, 5) uncontrolled hypertension (blood pressure (BP) ≥ 160/95 mm Hg), and e) uncontrolled diabetes (blood glucose (BG) ≥ 7.8 mmol/l).*	Page 6
4. Specify the rater population of interest (if applicable).	Not applicable to this paper	

5. Describe what is already known about reliability and agreement and provide a rationale for the study (if applicable).	Not reliability, but explains the purpose of the paper which addresses intra-individual variability in UIC, as follows: *Several methods exist to correct for intra-individual variation in population survey data. One method is to collect multiple days of data on each survey participant and average the data for each participant. This has substantial logistical costs when conducting a national survey. Another method is to apply a correction factor to the distribution.[8, 9] This requires estimating the correction factor, for example by collecting multiple samples from a representative subset of the survey population.* *This second method has been applied many times to dietary intake data [10-12] but has been applied less frequently to biochemical data.[13-15]*	Page 5, last para and page 6, 1st para
METHODS		
6. Explain how the sample size was chosen. State the determined number of raters, subjects/objects, and replicate observations.	This was a convenient sample, no power calculation performed. Of the 110 volunteers, 84 were eligible for inclusion. N = 84 – this is similar to another study that assessed variability in Australian indigenous adolescents (Mackerras et al. [10].). Replicate observations (UIC) numbered three urine collections, taken one week apart.	Page 6
7. Describe the sampling method.	Volunteers were recruited from a random selection of aged care facilities (independent, assisted and low care living) in the Illawarra region, south of Sydney in Australia. Exclusion criteria described in detail.	Page 6
8. Describe the measurement/rating process (e.g. time interval between repeated measurements, availability of clinical information, blinding).	Replicate observations (UIC) numbered three urine collections, taken one week apart. Protocol for urine collection clearly described. Batch analysis of urinary iodine by the accredited laboratory of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital (Sydney, NSW, Australia) described and coefficient of variation (CV) of the urinary iodine assay provided.	Page 7
9. State whether measurements/ratings were conducted independently.	Measurements were spot urine collections, same protocol followed for each collection and the same laboratory measured all samples. Accreditation of the laboratory for this analysis is described.	Page 7
10. Describe the statistical analysis.	*Descriptive analyses were performed using IBM SPSS(V19.0 IBM Corporation, Armonk NY). The 3-day repeated urinary iodine concentration estimates were used to determine a corrected UIC distribution after taking the natural logarithm of the iodine concentration. Repeated measures analysis of variance was performed to determine the between-person (sb) and total (sobs) standard deviations. A corrected UIC value was calculated for each person by adjusting the transformed value for each person, according to the following formula[15] using SAS (V9.2, SAS Cary, NC): Adjusted UIC = [(person's UIC – group mean) * (sb ÷ sobs)] + group mean. After exponentiation, the distribution of UICs was recalculated using the adjusted values and compared to the unadjusted mean values as well as the spot UIC collected on Day 1.*	Page 7, 2nd para
RESULTS		
11. State the actual number of raters and subjects/objects which were included and the number of replicate	See Table 2	Page 9

observations which were conducted		
12. Describe the sample characteristics of raters and subjects (e.g. training, experience)	Accreditation of the laboratory for this analysis is described.	Page 6, last para
13. Report estimates of reliability and agreement including measures of statistical uncertainty.	Estimates of reliability not appropriate to this study. Correct statistical results described as follows: *MUIC of the study population using the first spot urine collection indicated mild iodine deficiency (65.5 (IQR 42; 89)) µg/L). Correlations for transformed urinary iodine concentration values were: Days 1 and 2: r=0.48; p<0.01; Days 1 and 3: r=0.43; p<0.01; Days 2 and 3: r=0.41; p<0.01. The distribution of urinary iodine concentrations calculated by the different methods is shown in Table 2 and Figure 1. The s_b/s_{obs} was 0.83 when calculated using the three replicates and 0.79 when calculated using two replicates; i.e. the contraction in the distribution was slightly less with two replicates than three. Compared to the raw distribution, adjustment and averaging both reduced the spread of the distribution, especially at the upper end owing to the right skew in the data. Following adjustment using the three urine collections, the MUIC remained unchanged but the IQR was shifted upwards 65.2 (55; 94) while the upper end of the distribution (95th centile) changed from 176 to 136 µg/L. The percentage of participants with UIC ≥ 100 ug/L decreased from 21 % to 17 % following adjustment.*	Page 8, last para

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Intra-individual variation in urinary iodine concentrations: effect of adjustment on population distribution using two and three repeated spot urine collections

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Keywords: iodine, urinary iodine concentration, intra-individual variation, distribution

Abstract

Background: Iodine deficiency is assessed on a population level by comparing median spot urinary iodine concentrations (UIC) against references for pregnant and non-pregnant states. Intra-individual variation of a single UIC results in an overdispersed distribution and consequent errors in estimating the prevalence of deficiency and excess.

Methods: UIC data collected from 84 healthy volunteers, 60-95y from New South Wales, Australia, prior to the mandatory fortification programme, was used to determine the effect of adjustment for intra-individual variation on estimations of iodine deficiency and the population distribution. Three spot urine samples were collected, each one week apart. Repeated measures analysis of variance determined between-person (s_b) and total (s_{obs}) standard deviations. Adjusted UIC values were calculated as [(person's UIC – group mean) x (s_b/s_{obs})] + group mean, and a corrected UIC distribution calculated.

Results: The s_b/s_{obs} for using 3-samples and 2-samples was 0.83 and 0.79, respectively. Following adjustment for intra-individual variation, the proportion with UIC < 50 µg/L reduced from 33 % to 19%, while the proportion with UIC ≥ 100 µg/L changed from 21% to 17%. The 95th centile UIC decreased from 176 to 136 µg/L. Adjustment by taking averages yielded a lesser degree of contraction in the distribution than the analysis of variance method.

Conclusions: The addition of information about intra-individual variability has potential for increasing the interpretability of UIC data collected to monitor the iodine status of a population.

Summary

Article focus

- The iodine status of populations is defined by calculating median urinary iodine concentrations (UIC) from spot urine samples collected in surveys.
- High intra-individual (day-to-day) variation in urinary iodine excretion leads to an overdispersed distribution.
- Methods used in other areas of research to correct for intra-individual variation were applied to three repeated spot urine collections from a sample of older Australians to estimate a UIC distribution more reflective of long-term usual status.

Key messages

- Collection of a single urine sample leads to incorrect conclusions about the extremes of the population distribution of UIC.
- Adjustment using analysis of variance reduced the spread of the distribution more than calculating an average of each person's samples. Application of this method to surveillance of population iodine status and iodine fortification programmes could permit a more detailed assessment of the population and relevant subgroups.
- In this sample of older adults, three spot urine collections did not add value compared to two collections.

Strengths and limitations:

Older adults, who typically have a less varied diet than younger populations, were sampled therefore generalizability to other age groups may be limited.

Introduction

Iodine deficiency is one of the most common nutrient deficiencies in the world, with almost one billion people affected. Populations that consume diets that contain small amounts of fish and seafood, moderate to low quantities of milk and dairy products, and include locally produced fruits and vegetables grown in iodine-poor soils are likely to be iodine deficient. Iodine deficiency impacts across the life stages.[1] It affects growth and development (both cognitive and motor) during pregnancy, infancy and childhood. In older adults, iodine deficiency may play a role in declining cognitive function.[1] Consequently, good quality population-level data on iodine status is required to assess population status and design strategies which correct any deficiency but avoid introducing excessive intakes. The iodine status of populations is defined by calculating urinary iodine concentrations (UIC) from spot urine samples collected in a representative sample and comparing the median UIC (MUIC) against reference ranges.[2] Daily urinary excretion of iodine closely reflects iodine intake in non-pregnant populations therefore MUIC of a group is considered to be a valid biomarker of the status of that group.[3] However, the concentration measured in a single spot sample has large variation from day-to-day within individuals,[4-6]. This increases the spread of the distribution [7, 8] so that it does not reflect the range of long-term or 'usual' iodine status around the median in the population. It is the usual intake of iodine, not the intake on any one day that determines iodine status of groups. Consequently, a method to reduce or remove the effects of the measurement error due to the intra-individual variation that results from collecting a single spot urine sample from each survey participant would allow greater description of the population status.

Several methods exist to correct for intra-individual variation in population survey data. One method is to collect multiple days of data on each survey participant and average the data for each participant. This has substantial logistical costs when conducting a national survey.

Another method is to apply a correction factor to the distribution.[8, 9] This requires estimating the correction factor, for example by collecting multiple samples from a representative subset of the survey population

This second method has been applied many times to dietary intake data [10-12] but has been applied less frequently to biochemical data.[13-15] In a survey of indigenous Australian adolescents with MUIC of 51 µg/Lµg/L, correcting the distribution based on two urine samples per person, reduced the 95th centile from 129 µg/Lµg/L to 92 µg/Lµg/L.[13, 14] have highlighted the widespread misuse of calculating the proportions of UIC below the cut-off level of 100 µg/Lµg/L to estimate the prevalence of iodine deficiency. This will over- or underestimate the proportion with deficiency depending on the location of the median. In the previous example,[13] the raw data would be misinterpreted as showing that 90% were <100 µg/Lµg/L, whereas this was 97% after correction for within person variation. In clearly deficient populations, this difference is not important for program planning. However as a population approaches sufficiency, accurate estimation becomes more important for refining programs. Similarly in replete populations, an accurate estimate of the high intakes is needed to assess whether part of the population is reaching potentially adverse levels.

Remote indigenous people have a different lifestyle from that of urbanised non-Indigenous Australians. In this study, we investigate the intra-individual variation in UIC in older non-Indigenous Australians, living in an urban iodine deficient area prior to the introduction of mandatory fortification. We extend previous work by examining the impact of having two versus three samples for calculating the correction factor and compare this to the effect of averaging the results for each person.

Methods

Participants and recruitment

Between May and September 2009, 110 adults aged 60 - 95 years volunteered for a study that investigated the association between iodine status and cognition. English-speaking men and women were recruited from a random selection of aged care facilities (independent, assisted and low care living) in the Illawarra region, south of Sydney in Australia. Of the 110 participants, 84 subjects (25 men) met the study inclusion criteria and were enrolled. Twenty-six subjects were excluded due to: a) diagnosed dementia and/or Alzheimer's disease, b) cognitive decline as indicated by a Mini-Mental State Examination (MMSE score of <=23,[16] c) a previous stroke, d) current use of thyroxine or any other medications that may affect memory, 5) uncontrolled hypertension (blood pressure (BP) ≥ 160/95 mm Hg), and e) uncontrolled diabetes (blood glucose (BG) ≥ 7.8 mmol/l). The study protocol was approved by the Human Research Ethics Committee of the University of Wollongong and all participants provided written informed consent.

Weight (Tanita Scale, TBG622, Tanita Inc., Tokyo, Japan) and height (stadiometer) of subjects were measured. Body mass index was calculated as the ratio of weight (kg) divided by height squared (m^2). Nutritional status was assessed using the 18-item Mini Nutritional Assessment (MNA) which has been previously validated in older adults and classifies according to categories of well nourished, at-risk, or malnourished.[17] The Barthel index [18] was administered to assess ability to perform Activities of Daily Living, with a score of >50/100 indicating independence.

Biochemical data

Participants were provided with written instructions for spot urine sample collections, which included collection of the first voiding of the day, on the same day each week, where possible, over a three-week period. Urine samples were stored at -80°C and batch-analysed by the accredited laboratory of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital (Sydney, NSW, Australia). UIC was analysed using an adaptation of the Sandell-Kolthoff method using a ammonium persulphate digestion and microplate reading.[19] The coefficient of variation (CV) of the urinary iodine assay in the ICPMR laboratory is 16.7% at 46 ± 7.72 µg/L, 5.8% at 153 ± 8.9 µg/L, and 8.65% at 347 ± 30 µg/L. The group MUIC was compared to population-specific reference values.[2]

Statistical analyses

The UIC data were transformed using the natural logarithm to improve normality. Repeated measures analysis of variance was performed to determine the between-person (s_b) and total (s_{obs}) standard deviations. An adjusted log UIC value was calculated for each person as[20] : Adjusted UIC = [(person's day 1 UIC – group mean for day 1) * ($s_b \div s_{obs}$)] + group mean for day 1. (Equation 1)

The results were exponentiated. The adjustment procedure was performed twice using SAS (V9.2, SAS Cary, NC). First the correction factor (s_b/s_{obs}) was calculated using all three replicates, then it was calculated using only the first two replicates.

We also calculated the average for each person using all three replicates and for the first two replicates. Centiles of the distribution and the proportion below selected values were calculated for the raw Day 1 data and for distributions derived using adjustment or averaging. Descriptive analyses were performed using IBM SPSS (V19.0 IBM Corporation, Armonk NY).

Results

Sociodemographic characteristics of the study participants are shown in Table 1. All except four (5%) were classified as being well nourished according to the MNA classification, with the remainder in the "at risk" category. All participants were independently able to perform activities of daily living.

Table 1
Demographic and clinical characteristics of the study subjects (n = 84)

Characteristics	Subjects (*n* (%))
Sex (%)	
Men	25(30%)
Women	59(70%)
Age (years)	74 ± 8[a]
BMI[b]	
Mean	28.4 ± 4.7[a]
Underweight (< 18.5 kg/m^2)	0 (0%)
Normal Weight (18.5 - 24.9 kg/m^2)	16(19%)
Overweight (≥ 25 kg/m^2)	45 (54%)
Obese ≥ (30 kg/m^2)	23 (27%)
Education level	
≤ Year 12	39 (47%)
> Year 12	44 (52%)
Unknown	1 (1%)

[a] Mean ± standard deviation
[b] Calculated as kg/m^2

MUIC of the study population using the first spot urine collection indicated mild iodine deficiency (65.5 (IQR 42; 89)) µg/L). Correlations for transformed urinary iodine concentration values were: Days 1 and 2: r=0.48; p<0.01; Days 1 and 3: r=0.43; p<0.01; Days 2 and 3: r=0.41; p<0.01. The distribution of urinary iodine concentrations calculated by the different methods is shown in Table 2 and Figure 1. The s_b/s_{obs} was 0.83 when calculated using the three replicates and 0.79 when calculated using two replicates; i.e. the contraction in the distribution was slightly less with two replicates than three. Compared to the raw distribution, adjustment and averaging both reduced the spread of the distribution, especially

at the upper end owing to the right skew in the data. Following adjustment using the three urine collections, the MUIC remained unchanged but the IQR was shifted upwards 65.2 (55; 94) while the upper end of the distribution (95th centile) changed from 176 to 136 µg/L. The percentage of participants with UIC < 100 µg/L µg/L increased decreased from 79 % to 83 % following adjustment.

Table 2
Urinary iodine concentration distribution, raw data from one day, after adjustment for intra-individual variation of two and three spot sample collections, and averages of multiple collections

	Urinary iodine concentration distributions (µg/L)				
Centile	Raw data for Day 1	Average of Day 1 and Day 2	Day 1 corrected using 2 replicates	Average of Days 1, 2 and 3	Day 1 corrected using 3 replicates
5th	32.25	29.7	35.9	31.3	40.0
10th	35.50	35.5	40.8	38.3	43.0
25th	42.25	48.6	52.1	55.2	55.4
50th	65.5	65.5	66.8	69.0	65.2
75th	89.5	99.1	90.0	98.9	94.3
90th	123.5	146	115.8	138.8	118.0
95th	175.8	167.4	125.7	170.5	135.8
Maximum	340.0	278.5	204.8	222.0	167.9
Percent < 20 µg/Lµg/L	1%	0%	0%	0%	0%
Percent <50 µg/Lµg/L	33%	27%	21%	20%	19%
Percent < 100 µg/Lµg/L	79%	76%	82%	76%	83%

Taking an average of the three replicates also yielded similar results to taking an average of only two replicates. The averaging method contracted the distribution less than the adjustment method and, in particular had less effect in drawing the upper tail towards the median.

Discussion

In this population with a suboptimal iodine status, the collection of a single urine sample from each participant would lead to different conclusions about the extremes of the population distribution of UIC. This has also been shown previously in Indigenous Australian adolescents [13] and young Swiss women.[14]

In our population, there was no gain in having three, rather than two, samples as both adjustment factors were approximately 0.8 on the natural logarithmic scale. This means that the standard deviation of the final distribution is 80% of the original. The lower the ratio, the higher the correction - for example, a ratio of 0.5 would have resulted in a distribution with a standard deviation of half the width of the original.[21] We do not know whether having a greater number of replicates, such as seven or 14, or including different seasons, would have yielded the same result. Our urinary findings are consistent with the low variability in dietary iodine intake assessed at the same time in this population living in low level residential aged care facilities.[22] A similar adjustment ratio of 0.69 on the natural logarithmic scale for UIC has been reported in indigenous adolescents from the Darwin area, whose dietary patterns are limited in variety.[13] We hypothesize that the degree of adjustment would be larger in other populations that have more variety in food intake.

Heterogeneity in the iodine content of different foods and their frequency of consumption in different populations will affect the magnitude of intra-individual variability in UIC. This suggests that the intra-individual variability in UIC would change after a fortification

program is introduced. It could be further hypothesised that the intra-individual variability might increase if one or a small number of foods is fortified, particularly at high concentrations. In Tasmania, the interquartile range widened as the MUIC value increased following iodisation of salt (25-65g iodine/kg salt) used in bread. Pre-fortification, MUIC in schoolchildren was 73 (IQR 56-100) µg/L, which increased to 108 (73-158) µg/L following voluntary fortification, and to 132µg/L (96-198) µg/L) post mandatory fortification.[23] However, it is not possible to compare these results to studies which report other parameters for the UIC distribution such as the range [14] or the 10-90th centiles.[24] It is less clear whether variability would increase or decrease if a wide range of foods are fortified and/or at a lower concentration. We hypothesise that adjustment factors need to be reassessed if iodine intake changes and multiple factors might be needed if there is geographical variation in iodine status within a country.

The greater narrowing in population distribution that results from the adjustment method, compared to the averaging method, is expected if multiple days are needed to estimate the long-term UIC for an individual. This is because the average of few days of urinary collection would still contain intra-individual variation. However, studies that collect a greater number of replicate samples than in the current study are still needed to compare the distribution determined by statistical adjustment to a directly assessed long-term average UIC. Another consideration is the use of spot urine samples as a proxy for assessment of iodine status on a population level. A spot sample does not reflect intake over an entire day for which a 24-hour collection would be needed. Konig et al [25] have reported a trend for higher intra-individual variation for spot UIC (38 %) versus measured 24-hour urinary iodine excretion (32 %),

Our study sample comprised older adults, an age group who have been studied least for iodine status, and who also often have impaired renal function. We have previously suggested that spot urinary iodine concentrations may be under-estimating 24 hr excretion in this study population [22] but this would not impact on intra-individual variability of UIC which is the topic of the current paper. Any variation in day-to-day fluid intake would be included in the changing UIC for each person in the study on each day of collection. However, as the samples were all collected within a three week period, we would not have included any variation in fluid intake related to seasons. This may have underestimated the degree of adjustment in this population. However a reduction in total fluid intake, and therefore urine volume, might or might not affect the day-to-day variation.

In our study, we had replicate samples on all participants. A more logistically feasible alternative in a large survey is to collect the replicates in a representative sub-set and apply the adjustment factor calculated in the sub-set to the whole population. It may be necessary to subdivide the population, for example, by age and sex, and ensure that there are enough participants in each sub-division to permit a suitable range of adjustment factors to be calculated. The method we have used [20] is the simplest of several methods that have a similar purpose.[26] The disadvantage of collecting replicates in a sub-set only is that, although an estimate of the usual UIC distribution of the population is obtained, the method treats each individual as representative of a larger group and so the value calculated for each individual in Equation 1 is theoretical. Therefore, if it is desired to link intake with excretion at an individual level, then replicate information about both intake and excretion for each survey participant would be preferable but it is also possible to correct a regression coefficient for within person variability.[27] In addition, the method we used assumes that the data can be normalised. If this is not true, then alternatives include calculating an average

for each participant [28] or using a complex method such as the National Cancer Institute method.[29]

Conclusion

In a sample of healthy older Australian adults who were iodine deplete, the use of two or three spot urine samples for adjustment of intra-individual variation in urinary iodine concentration resulted in a narrowed population distribution, particularly at the upper end. Statistical adjustment yielded a stronger correction than averaging the replicates. There was no important gain in collecting a third sample in this population; however this finding might be specific to our group and not generalizable to other age groups. The impact of the adjustment in narrowing the distribution would be greater in groups with more varied dietary intakes, and therefore wider intra-individual variation in UIC. These results provide a case for further work to investigate the usefulness of determining adjustment factors to remove intra-individual variability as part of population assessment of iodine status.

Figure legend:

Figure 1 Population distribution of urinary iodine, according to number of days of spot urine collection

Contribution of each author:

Karen E Charlton: Conceptualisation of study design, drafting initial manuscript

Marijka J Batterham: Statistical analysis, editing of manuscript.

Li Min Tan Buchanan: Participant recruitment, data collection, data entry, editing of manuscript.

Dorothy Mackerras: Statistical interpretation, editing of manuscript.

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Competing Interests

None

Data sharing

No additional data available.

References

1. Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. Lancet. 2008;**372**:1251-62.

2. World Health Organization. Assessment of iodine deficiency disorders and monitoring their elimination. 3rd Edition ed. Geneva: WHO; 2007.

3. Ristic-Medic D, Piskackova Z, Hooper L, et al. Methods of assessment of iodine status in humans: a systematic review. Am J Clin Nutr. 2009;**89**:2052S-69S.

4. Rasmussen LB, Ovesen L, Christiansen E. Day-to-day and within-day variation in urinary iodine excretion. Eur J Clin Nutr. 1999;**53**:401-7.

5. Rasmussen LB, Ovesen L, Bulow I, et al. Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. Br J Nutr. 2002;**87**:61-9.

6. Als C, Helbling A, Peter K, et al. Urinary iodine concentration follows a circadian rhythm: A study with 3023 spot urine samples in adults and children. J Clin Endocrinol Metab. 2000;**85**:1367-9.

7. Andersen S, Karmisholt J, Pedersen KM, et al. Reliability of studies of iodine intake and recommendations for number of samples in groups and in individuals. Br J Nutr. 2008;**99**:813-8.

8. Armstrong BK, White E, Saracci R. Principles of exposure measurement in epidemiology. Oxford: Oxford University Press; 1992.

9. Dyer AR, Shipley M, Elliott P. Urinary electrolyte excretion in 24 hours and blood-pressure in the Intersalt study. 1. Estimates of reliability Am J Epi. 1994;**139**:927-39.

10. Subar AF, Dodd KW, Guenther PM, et al. The Food Propensity Questionnaire: Concept, Development, and Validation for Use as a Covariate in a Model to Estimate Usual Food Intake. J Am Diet Assoc. 2006;**106**:1556-63.

11. Freedman LS, Midthune D, Carroll RJ, et al. Adjustments to improve the estimation of usual dietary intake distributions in the population. J Nutr. 2004;**134**:1836-43.

12. Kipnis V, Midthune D, Buckman DW, et al. Modeling Data with Excess Zeros and Measurement Error: Application to Evaluating Relationships between Episodically Consumed Foods and Health Outcomes. biometrics. 2009;**65**:1003-10.

13. Mackerras DEM, Singh GR, Eastman CJ. Iodine status of Aboriginal teenagers in the Darwin region before mandatory iodine fortification of bread. Med J Aust. 2011;**194**:126-30.

14. Zimmermann MB, Andersson M. Assessment of iodine nutrition in populations: past, present, and future. Nutr Rev. 2012;**70**:553-70.

15. Looker AC, Dallman PR, Carroll MD, et al. Prevalence of iron deficiency in the United States. JAMA. 1997;**277**:973-6.

16. Folstein MF, Folstein SE, McHugh PR. Mini-Mental State - practical method for grading cognitive state of patients for clinician J Psych Res. 1975;**12**:189-98.

17. Guigoz Y, Vellas B, Garry PJ. Mini nutritional assessment : A practical assessment tool for grading the nutritional state of elderly patients. Facts Res Gerontol. 1994;**4**:15-59.

18. Mahoney F, Barthel D. Functional evaluation: The Barthel Index. Maryland State Med J. 1965;**14**:56-61.

19. Ohashi T, Yamaki M, Pandav CS, et al. Simple microplate method for determination of urinary iodine. Clin Chem. 2000;**46**:529-36.

20. Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Reference SCotSEoD, Intakes. Dietary Reference Intakes: Applications in Dietary Planning. Washington, DC: The National Academies Press; 2003.

21. Baines J, Cunningham J, Leemhuis C, et al. Risk Assessment to Underpin Food Regulatory Decisions: An Example of Public Health Nutritional Epidemiology. Nutrients. 2011;**3**:164-85.

22. Tan L, Charlton KE, Roodenrys S, et al. Validation of an iodine-specific Food Frequency Questionnaire to estimate dietary iodine intake in older Australians. Nutr Diet. 2013;**70**:71-8.

23. Depaoli K, Seal JA, Taylor R, editors. Progressive improvements in iodine status in Tasmania with voluntary then mandatory foritifcation: Preliminary results from the 2011 Tasmanian Urinary Iodine Survey. Nutrition & Dietetics; 2012: Blackwell Publishing Asia.

24. U.S. Centers for Disease Control and Prevention. Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population 2012. Atlanta, GA.: National Center for Environmental Health; 2012.

25. Konig F, Andersson M, Hotz K, et al. Ten Repeat Collections for Urinary Iodine from Spot Samples or 24-Hour Samples Are Needed to Reliably Estimate Individual Iodine Status in Women. J Nutr. 2011;**141**:2049-54.

26. National Cancer Institute. Usual Dietary Intakes: SAS macros for analysis of a single dietary component: http://riskfactor.cancer.gov/diet/usualintakes/macros_single.html.; 2010.

27. Sempos CT, Johnson NE, Smith EL, et al. Effects of intraindividual and interindividual variation in repeated dietary records. Am J Epi. 1985;**121**:120-30.

28. Food Standards Australia New Zealand (FSANZ). The 2007 Australian National Children's Nutrition and Physical Activity Survey Volume Eight: Dietary exposure to food sourced chemicals. Canberra: Department of Health and Ageing; 2012.

29. Tooze JA, Kipnis V, Buckman DW, et al. A mixed-effects model approach for estimating the distribution of usual intake of nutrients: The NCI method. Stat Med. 2010;**29**:2857-68.

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Keywords: iodine, urinary iodine concentration, intra-individual variation, distribution

Introduction

Iodine deficiency is one of the most common nutrient deficiencies in the world, with almost one billion people affected. Populations that consume diets that contain small amounts of fish and seafood, moderate to low quantities of milk and dairy products, and include locally produced fruits and vegetables grown in iodine-poor soils are likely to be iodine deficient. Iodine deficiency impacts across the life stages.[1] It affects growth and development (both cognitive and motor) during pregnancy, infancy and childhood. In older adults, iodine deficiency may play a role in declining cognitive function.[1] Consequently, good quality population-level data on iodine status is required to assess population status and design strategies which correct any deficiency but avoid introducing excessive intakes. The iodine status of populations is defined by calculating urinary iodine concentrations (UIC) from spot urine samples collected in a representative sample and comparing the median UIC (MUIC) against reference ranges.[2] Daily urinary excretion of iodine closely reflects iodine intake in non-pregnant populations therefore MUIC of a group is considered to be a valid biomarker of the status of that group.[3] However, the concentration measured in a single spot sample has large variation from day-to-day within individuals,[4-6]. This increases the spread of the distribution [7, 8] so that it does not reflect the range of long-term or 'usual' iodine status around the median in the population. It is the usual intake of iodine, not the intake on any one day that determines iodine status of groups. Consequently, a method to reduce or remove the effects of the measurement error due to the intra-individual variation that results from collecting a single spot urine sample from each survey participant would allow greater description of the population status.

Several methods exist to correct for intra-individual variation in population survey data. One method is to collect multiple days of data on each survey participant and average the data for each participant. This has substantial logistical costs when conducting a national survey.

Another method is to apply a correction factor to the distribution.[8, 9] This requires estimating the correction factor, for example by collecting multiple samples from a representative subset of the survey population

This second method has been applied many times to dietary intake data [10-12] but has been applied less frequently to biochemical data.[13-15] In a survey of indigenous Australian adolescents with MUIC of 51 µg/L~~ug/L~~µg/L, correcting the distribution based on two urine samples per person, reduced the 95th centile from 129 µg/L~~ug/L~~µg/L to 92 µg/L~~ug/L~~µg/L.[13, 14] have highlighted the widespread misuse of calculating the proportions of UIC below the cut-off level of 100 µg/L~~ug/L~~µg/L to estimate the prevalence of iodine deficiency. This will over- or underestimate the proportion with deficiency depending on the location of the median. In the previous example,[13] the raw data would be misinterpreted as showing that 90% were <100 µg/L~~ug/L~~µg/L, whereas this was 97% after correction for within person variation. In clearly deficient populations, this difference is not important for program planning. However as a population approaches sufficiency, accurate estimation becomes more important for refining programs. Similarly in replete populations, an accurate estimate of the high intakes is needed to assess whether part of the population is reaching potentially adverse levels.

Remote indigenous people have a different lifestyle from that of urbanised non-Indigenous Australians. In this study, we investigate the intra-individual variation in UIC in older non-Indigenous Australians, living in an urban iodine deficient area prior to the introduction of mandatory fortification. We extend previous work by examining the impact of having two versus three samples for calculating the correction factor and compare this to the effect of averaging the results for each person.

Methods

Participants and recruitment

Between May and September 2009, 110 ~~English-speaking men and women~~ adults aged 60 - 95 years volunteered for a study that investigated the association between iodine status and cognition. English-speaking men and women ~~Volunteers~~ were recruited from a random selection of aged care facilities (independent, assisted and low care living) in the Illawarra region, south of Sydney in Australia. Of the 110 participants, 84 subjects (25 men) met the study inclusion criteria and were enrolled. Twenty-six subjects were excluded due to: a) diagnosed dementia and/or Alzheimer's disease, b) cognitive decline as indicated by a Mini-Mental State Examination (MMSE[9] score of <=23,[16] c) a previous stroke, d) current use of thyroxine or any other medications that may affect memory, 5) uncontrolled hypertension (blood pressure (BP) ≥ 160/95 mm Hg), and e) uncontrolled diabetes (blood glucose (BG) ≥ 7.8 mmol/l). The study protocol was approved by the Human Research Ethics Committee of the University of Wollongong and all participants provided written informed consent.

Weight (Tanita Scale, TBG622, Tanita Inc., Tokyo, Japan) and height (stadiometer) of subjects were measured. Body mass index was calculated as the ratio of weight (kg) divided by height squared (m^2). Nutritional status was assessed using the 18-item Mini Nutritional Assessment (MNA) which has been previously validated in older adults and classifies according to categories of well nourished, at-risk, or malnourished.[17] The Barthel index [18] was administered to assess ability to perform Activities of Daily Living, with a score of >50/100 indicating independence.

Biochemical data

Participants were provided with written instructions for spot urine sample collections, which included collection of the first voiding of the day, on the same day each week, where possible, over a three-week period. Urine samples were stored at -80°C and batch-analysed by the accredited laboratory of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital (Sydney, NSW, Australia). UIC was analysed using an adaptation of the Sandell-Kolthoff method using a ammonium persulphate digestion and microplate reading.[19] The coefficient of variation (CV) of the urinary iodine assay in the ICPMR laboratory is 16.7% at 46 ± 7.72 µg/L, 5.8% at 153 ± 8.9 µg/L, and 8.65% at 347 ± 30 µg/L. The group MUIC was compared to population-specific reference values.[2]

Statistical analyses

The UIC data were transformed using the natural logarithm to improve normality. Repeated measures analysis of variance was performed to determine the between-person (s_b) and total (s_{obs}) standard deviations. An adjusted log UIC value was calculated for each person as[20] : Adjusted UIC = [(person's day 1 UIC – group mean for day 1) * ($s_b \div s_{obs}$)] + group mean for day 1. (Equation 1)

The results were exponentiated. The adjustment procedure was performed twice using SAS (V9.2, SAS Cary, NC). First the correction factor (s_b/s_{obs}) was calculated using all three replicates, then it was calculated using only the first two replicates.

We also calculated the average for each person using all three replicates and for the first two replicates. Centiles of the distribution and the proportion below selected values were calculated for the raw Day 1 data and for distributions derived using adjustment or averaging. Descriptive analyses were performed using IBM SPSS (V19.0 IBM Corporation, Armonk NY).

Results

Sociodemographic characteristics of the study participants are shown in Table 1. All except four (5%) were classified as being well nourished according to the MNA classification, with the remainder in the "at risk" category. All participants were independently able to perform activities of daily living.

Table 1
Demographic and clinical characteristics of the study subjects (n = 84)

Characteristics	Subjects (*n* =84) (%))
Sex (%)	
Men	25(30%)
Women	59(70%)
Age (years)	74 ± 8[a]
BMI[b]	
Mean	28.4 ± 4.7[a]
Underweight (< 18.5 kg/m^2)	0 (0%)
Normal Weight (18.5 - 24.9 kg/m^2)	16(19%)
Overweight (≥ 25 kg/m^2)	45 (54%)
Obese ≥ (30 kg/m^2)	23 (27%)
MNA score	
Mean	27.2 ± 3.6[a]
Malnourished (< 17 points)	0 (0%)
At risk of Malnutrition (17 - 23.5 points)	4 (5%)
Well-nourished (≥ 24 points)	78 (93%)
Unknown	2 (2%)
Barthel Index[17]	
Able to independently perform activities of daily living (>50 points)	84 (100%)
MMSE score[18]	
0 to 23	0 (0%)
24-30	83 (99%)
Unknown	1 (1%)

Education level	
≤ Year 12	39 (47%)
> Year 12	44 (52%)
Unknown	1 (1%)

[a] Mean ± standard deviation
[b] Calculated as kg/m^2

MUIC of the study population using the first spot urine collection indicated mild iodine deficiency (65.5 (IQR 42; 89)) µg/L). Correlations for transformed urinary iodine concentration values were: Days 1 and 2: r=0.48; p<0.01; Days 1 and 3: r=0.43; p<0.01; Days 2 and 3: r=0.41; p<0.01. The distribution of urinary iodine concentrations calculated by the different methods is shown in Table 2 and Figure 1. The s_b/s_{obs} was 0.83 when calculated using the three replicates and 0.79 when calculated using two replicates; i.e. the contraction in the distribution was slightly less with two replicates than three. Compared to the raw distribution, adjustment and averaging both reduced the spread of the distribution, especially at the upper end owing to the right skew in the data. Following adjustment using the three urine collections, the MUIC remained unchanged but the IQR was shifted upwards 65.2 (55; 94) while the upper end of the distribution (95th centile) changed from 176 to 136 µg/L. The percentage of participants with UIC ~~<~~≤ 100 µg/L ~~ug/L~~µg/L increased decreased from 792~~1~~ % to 83~~17~~ % following adjustment.

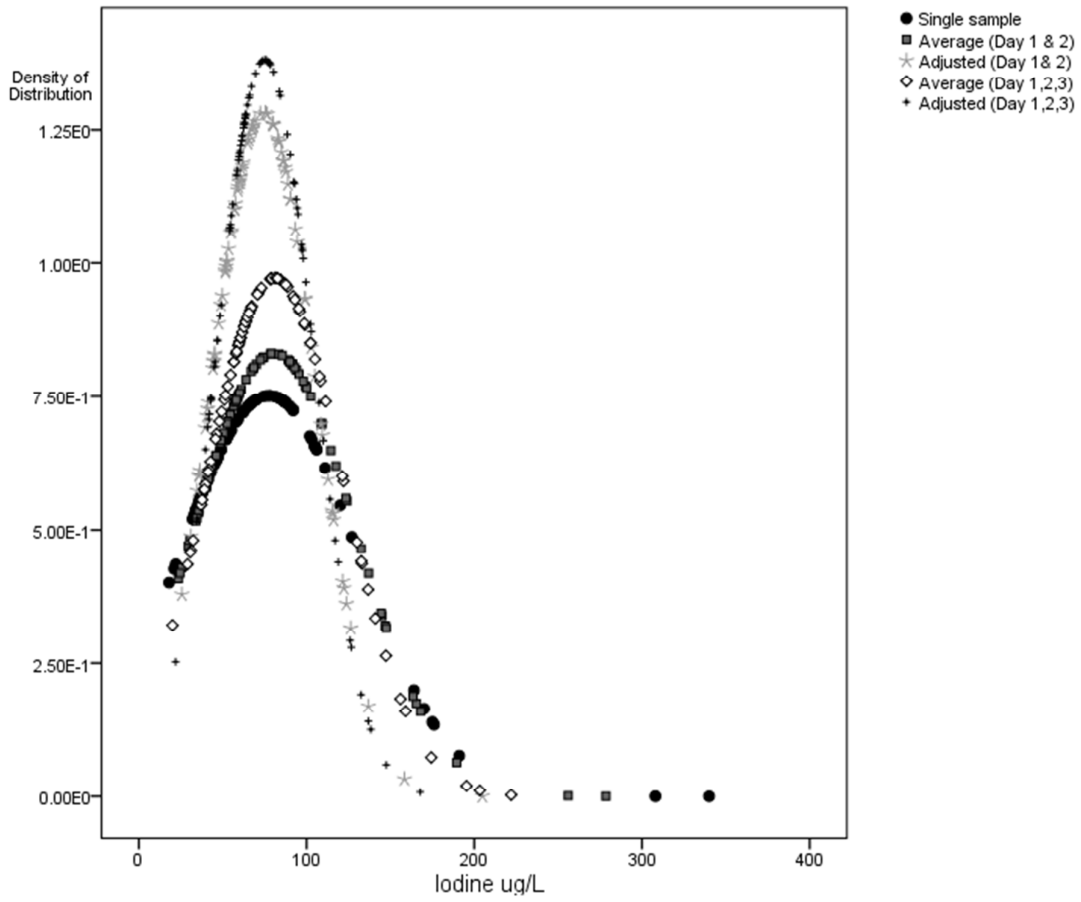
Table 2
Urinary iodine concentration distribution, raw data from one day, after adjustment for intra-individual variation of two and three spot sample collections, and averages of multiple collections

	Urinary iodine concentration distributions (~~ug/L~~µg/L)				
Centile	Raw data for Day 1	Average of Day 1 and Day 2	Day 1 corrected using 2 replicates	Average of Days 1, 2 and 3	Day 1 corrected using 3 replicates
5th	32.25	29.7	35.9	31.3	40.0
10th	35.50	35.5	40.8	38.3	43.0
25th	42.25	48.6	52.1	55.2	55.4
50th	65.5	65.5	66.8	69.0	65.2

75th	89.5	99.1	90.0	98.9	94.3
90th	123.5	146	115.8	138.8	118.0
95th	175.8	167.4	125.7	170.5	135.8
Maximum	340.0	278.5	204.8	222.0	167.9
Percent < 20 µg/Lug/Lµg/L	1%	0%	0%	0%	0%
Percent <50 µg/Lug/Lµg/L	33%	27%	21%	20%	19%
Percent <= 100 µg/Lug/Lµg/L	7921%	7624%	8218%	7624%	8317%

Taking an average of the three replicates also yielded similar results to taking an average of only two replicates. The averaging method contracted the distribution less than the adjustment method and, in particular had less effect in drawing the upper tail towards the median.

Figure 1 Population distribution of urinary iodine, according to number of days of spot urine collection



Discussion

In this population with a suboptimal iodine status, the collection of a single urine sample from each participant would lead to different conclusions about the extremes of the population distribution of UIC. This has also been shown previously in Indigenous Australian adolescents [13] and young Swiss women.[14]

In our population, there was no gain in having three, rather than two, samples as both adjustment factors were approximately 0.8 on the natural logarithmic scale. This means that the standard deviation of the final distribution is 80% of the original. The lower the ratio, the higher the correction - for example, a ratio of 0.5 would have resulted in a distribution with a

standard deviation of half the width of the original.[21] We do not know whether having a greater number of replicates, such as seven or 14, or including different seasons, would have yielded the same result. Our urinary findings are consistent with the low variability in dietary iodine intake assessed at the same time in this population living in low level residential aged care facilities.[22] A similar adjustment ratio of 0.69 on the natural logarithmic scale for UIC has been reported in indigenous adolescents from the Darwin area, whose dietary patterns are limited in variety.[13] We hypothesize that the degree of adjustment would be larger in other populations that have more variety in food intake.

Heterogeneity in the iodine content of different foods and their frequency of consumption in different populations will affect the magnitude of intra-individual variability in UIC. This suggests that the intra-individual variability in UIC would change after a fortification program is introduced. It could be further hypothesised that the intra-individual variability might increase if one or a small number of foods is fortified, particularly at high concentrations. In Tasmania, the interquartile range widened as the MUIC value increased following iodisation of salt (25-65g iodine/kg salt) used in bread. Pre-fortification, MUIC in schoolchildren was 73 (IQR 56-100) µg/L, which increased to 108 (73-158) µg/L following voluntary fortification, and to 132µg/L (96-198) µg/L) post mandatory fortification.[23] However, it is not possible to compare these results to studies which report other parameters for the UIC distribution such as the range [14] or the 10-90th centiles.[24] It is less clear whether variability would increase or decrease if a wide range of foods are fortified and/or at a lower concentration. We hypothesise that adjustment factors need to be reassessed if iodine intake changes and multiple factors might be needed if there is geographical variation in iodine status within a country.

The greater narrowing in population distribution that results from the adjustment method, compared to the averaging method, is expected if multiple days are needed to estimate the long-term UIC for an individual. This is because the average of few days of urinary collection would still contain intra-individual variation. However, studies that collect a greater number of replicate samples than in the current study are still needed to compare the distribution determined by statistical adjustment to a directly assessed long-term average UIC. Another consideration is the use of spot urine samples as a proxy for assessment of iodine status on a population level. A spot sample does not reflect intake over an entire day for which a 24-hour collection would be needed. Konig et al [25] have reported a trend for higher intra-individual variation for spot UIC (38 %) versus measured 24-hour urinary iodine excretion (32 %),

Our study sample comprised older adults, an age group who have been studied least for iodine status, and who also often have impaired renal function. We have previously suggested that spot urinary iodine concentrations may be under-estimating 24 hr excretion in this study population [22] but this would not impact on intra-individual variability of UIC which is the topic of the current paper. Any variation in day-to-day fluid intake would be included in the changing UIC for each person in the study on each day of collection. However, as the samples were all collected within a three week period, we would not have included any variation in fluid intake related to seasons. This may ~~would~~ have underestimated the degree of adjustment in this population. However a reduction in total fluid intake, and therefore urine volume, might or might not affect the day-to-day variation. In our study, we had replicate samples on all participants. A more logistically feasible alternative in a large survey is to collect the replicates in a representative sub-set and apply the adjustment factor calculated in the sub-set to the whole population. It may be necessary to subdivide the population, for example, by age and sex, and ensure that there are enough

participants in each sub-division to permit a suitable range of adjustment factors to be calculated. The method we have used [20] is the simplest of several methods that have a similar purpose.[26] The disadvantage of collecting replicates in a sub-set only is that, although an estimate of the usual UIC distribution of the population is obtained, the method treats each individual as representative of a larger group and so the value calculated for each individual in Equation 1 is theoretical. Therefore, if it is desired to link intake with excretion at an individual level, then replicate information about both intake and excretion for each survey participant would be preferable but it is also possible to correct a regression coefficient for within person variability.[27] In addition, the method we used assumes that the data can be normalised. If this is not true, then alternatives include calculating an average for each participant [28] or using a complex method such as the National Cancer Institute method.[29]

Conclusion

In a sample of healthy older Australian adults who were iodine deplete, the use of two or three spot urine samples for adjustment of intra-individual variation in urinary iodine concentration resulted in a narrowed population distribution, particularly at the upper end. Statistical adjustment yielded a stronger correction than averaging the replicates. There was no important gain in collecting a third sample in this population; however this finding might be specific to our group and not generalizable to other age groups. The impact of the adjustment in narrowing the distribution would be greater in groups with more varied dietary intakes, and therefore wider intra-individual variation in UIC. These results provide a case for further work to investigate the usefulness of determining adjustment factors to remove intra-individual variability as part of population assessment of iodine status.

References

1. Zimmermann MB, Jooste PL, Pandav CS. Iodine-deficiency disorders. Lancet. 2008;**372**:1251-62.

2. World Health Organization. Assessment of iodine deficiency disorders and monitoring their elimination. 3rd Edition ed. Geneva: WHO; 2007.

3. Ristic-Medic D, Piskackova Z, Hooper L, Ruprich J, Casgrain A, Ashton K, et al. Methods of assessment of iodine status in humans: a systematic review. Am J Clin Nutr. 2009;**89**:2052S-69S.

4. Rasmussen LB, Ovesen L, Christiansen E. Day-to-day and within-day variation in urinary iodine excretion. Eur J Clin Nutr. 1999;**53**:401-7.

5. Rasmussen LB, Ovesen L, Bulow I, Jorgensen T, Knudsen N, Laurberg P, et al. Dietary iodine intake and urinary iodine excretion in a Danish population: effect of geography, supplements and food choice. Br J Nutr. 2002;**87**:61-9.

6. Als C, Helbling A, Peter K, Haldimann I, Zimmerli B, Gerber H. Urinary iodine concentration follows a circadian rhythm: A study with 3023 spot urine samples in adults and children. J Clin Endocrinol Metab. 2000;**85**:1367-9.

7. Andersen S, Karmisholt J, Pedersen KM, Laurberg P. Reliability of studies of iodine intake and recommendations for number of samples in groups and in individuals. Br J Nutr. 2008;**99**:813-8.

8. Armstrong BK, White E, Saracci R. Principles of exposure measurement in epidemiology. Oxford: Oxford University Press; 1992.

9. Dyer AR, Shipley M, Elliott P. Urinary electrolyte excretion in 24 hours and blood-pressure in the Intersalt study. 1. Estimates of reliability Am J Epi. 1994;**139**:927-39.

10. Subar AF, Dodd KW, Guenther PM, Kipnis V, Midthune D, McDowell M, et al. The Food Propensity Questionnaire: Concept, Development, and Validation for Use as a Covariate in a Model to Estimate Usual Food Intake. J Am Diet Assoc. 2006;**106**:1556-63.

11. Freedman LS, Midthune D, Carroll RJ, Krebs-Smith S, Subar AF, Troiano RP, et al. Adjustments to improve the estimation of usual dietary intake distributions in the population. J Nutr. 2004;**134**:1836-43.

12. Kipnis V, Midthune D, Buckman DW, Dodd KW, Guenther PM, Krebs-Smith SM, et al. Modeling Data with Excess Zeros and Measurement Error: Application to Evaluating Relationships between Episodically Consumed Foods and Health Outcomes. biometrics. 2009;**65**:1003-10.

13. Mackerras DEM, Singh GR, Eastman CJ. Iodine status of Aboriginal teenagers in the Darwin region before mandatory iodine fortification of bread. Med J Aust. 2011;**194**:126-30.

14. Zimmermann MB, Andersson M. Assessment of iodine nutrition in populations: past, present, and future. Nutr Rev. 2012;**70**:553-70.

15. Looker AC, Dallman PR, Carroll MD, Gunter EW, Johnson CL. Prevalence of iron deficiency in the United States. JAMA. 1997;**277**:973-6.

16. Folstein MF, Folstein SE, McHugh PR. Mini-Mental State - practical method for grading cognitive state of patients for clinician J Psych Res. 1975;**12**:189-98.

17. Guigoz Y, Vellas B, Garry PJ. Mini nutritional assessment : A practical assessment tool for grading the nutritional state of elderly patients. Facts Res Gerontol. 1994;**4**:15-59.

18. Mahoney F, Barthel D. Functional evaluation: The Barthel Index. Maryland State Med J. 1965;**14**:56-61.

19. Ohashi T, Yamaki M, Pandav CS, Karmarkar MG, Irie M. Simple microplate method for determination of urinary iodine. Clin Chem. 2000;**46**:529-36.

20. Subcommittee on Interpretation and Uses of Dietary Reference Intakes, Reference SCotSEoD, Intakes. Dietary Reference Intakes: Applications in Dietary Planning. Washington, DC: The National Academies Press; 2003.

21. Baines J, Cunningham J, Leemhuis C, Hambridge T, Mackerras D. Risk Assessment to Underpin Food Regulatory Decisions: An Example of Public Health Nutritional Epidemiology. Nutrients. 2011;**3**:164-85.

22. Tan L, Charlton KE, Roodenrys S, Ma G, Batterham M. Validation of an iodine-specific Food Frequency Questionnaire to estimate dietary iodine intake in older Australians. Nutr Diet. 2013;**70**:71-8.

23. Depaoli K, Seal JA, Taylor R, editors. Progressive improvements in iodine status in Tasmania with voluntary then mandatory foritifcation: Preliminary results from the 2011 Tasmanian Urinary Iodine Survey. Nutrition & Dietetics; 2012: Blackwell Publishing Asia.

24. U.S. Centers for Disease Control and Prevention. Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population 2012. Atlanta, GA.: National Center for Environmental Health; 2012.

25. Konig F, Andersson M, Hotz K, Aeberli I, Zimmermann MB. Ten Repeat Collections for Urinary Iodine from Spot Samples or 24-Hour Samples Are Needed to Reliably Estimate Individual Iodine Status in Women. J Nutr. 2011;**141**:2049-54.

26. National Cancer Institute. Usual Dietary Intakes: SAS macros for analysis of a single dietary component: http://riskfactor.cancer.gov/diet/usualintakes/macros_single.html.; 2010.

27. Sempos CT, Johnson NE, Smith EL, Gilligan CE. Effects of intraindividual and interindividual variation in repeated dietary records. Am J Epi. 1985;**121**:120-30.

28. Food Standards Australia New Zealand (FSANZ). The 2007 Australian National Children's Nutrition and Physical Activity Survey Volume Eight: Dietary exposure to food sourced chemicals. Canberra: Department of Health and Ageing; 2012.

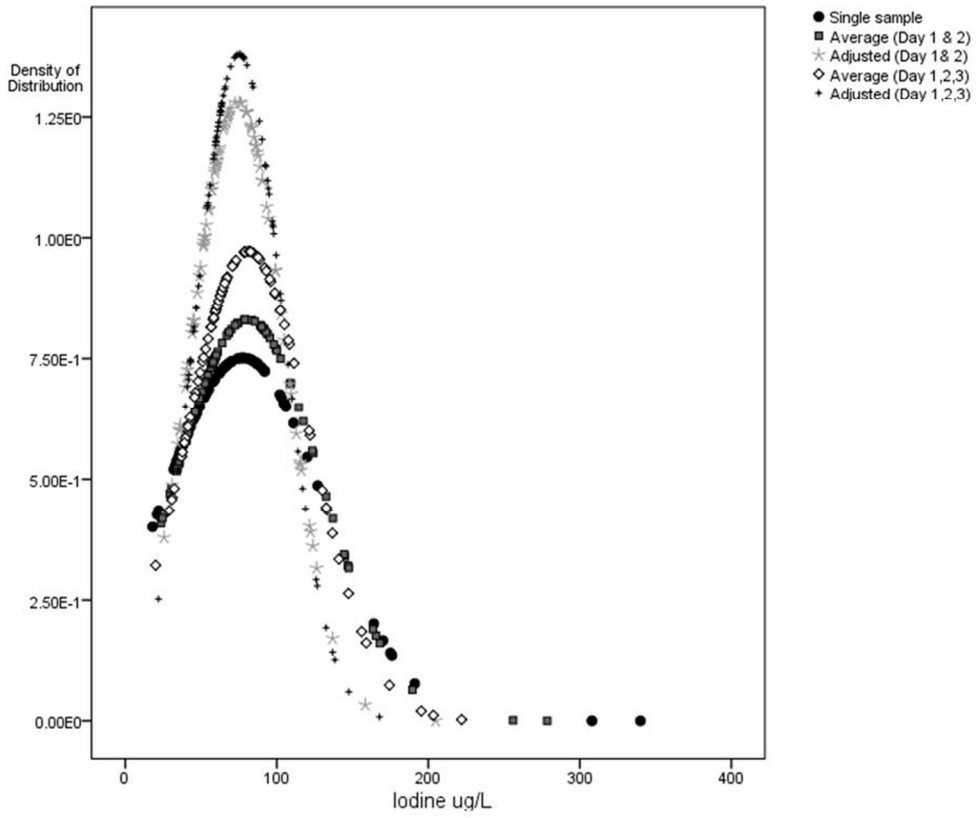
29. Tooze JA, Kipnis V, Buckman DW, Carroll RJ, Freedman LS, Guenther PM, et al. A mixed-effects model approach for estimating the distribution of usual intake of nutrients: The NCI method. Stat Med. 2010;**29**:2857-68.

This paper is not a reliability study but we have used the Guidelines for Reporting Reliability and Agreement Studies (GRRAS) checklist as this seems to be the closest in terms of study design.

		Page in manuscript where information appears
TITLE AND ABSTRACT		
1. Identify in title or abstract that interrater/intrarater reliability or agreement was investigated.	Title reflects the analysis performed" **Intra-individual variation in urinary iodine concentrations: Effect of adjustment on population distribution using two and three repeated spot urine collections .** "Abstract states "UIC data collected from 84 healthy volunteers, 60-95y from New South Wales, Australia, prior to the mandatory fortification programme was used to determine the effect of adjustment for intra-individual variation on estimations of iodine deficiency and the population distribution."	Page 1 and 2
INTRODUCTION		
2. Name and describe the diagnostic or measurement device of interest explicitly	Measurement is repeated spot urine samples for analysis of Urinary Iodine Concentration (UIC). This is explained in detail, and the methodological problems associated with the single spot urine (currently the recommended method for population-level assessment of iodine status).	Pages 4 (2nd para),5, and 6
3. Specify the subject population of interest.	This is adequately described as follows: *Remote indigenous people have a different lifestyle from that of urbanised non-Indigenous Australians. In this study, we investigate the intra-individual variation in UIC in older non-Indigenous Australians, living in an urban iodine deficient area prior to the introduction of mandatory fortification. We extend previous work by examining the impact of having two versus three samples for calculating the correction factor and compare this to the effect of averaging the results for each person.* *Between May and September 2009, 110 English-speaking men and women aged 60 - 95 years volunteered for a study that investigated the association between iodine status and cognition. Volunteers were recruited from a random selection of aged care facilities (independent, assisted and low care living) in the Illawarra region, south of Sydney in Australia. Of the 110 participants, 84 subjects (25 men) met the study inclusion criteria and were enrolled. Twenty-six subjects were excluded due to: a) diagnosed dementia and/or Alzheimer's disease, b) cognitive decline as indicated by a Mini-Mental State Examination (MMSE score of <=23,[16] c) a previous stroke, d) current use of thyroxine or any other medications that may affect memory, 5) uncontrolled hypertension (blood pressure (BP) ≥ 160/95 mm Hg), and e) uncontrolled diabetes (blood glucose (BG) ≥ 7.8 mmol/l).*	Page 6
4. Specify the rater population of interest (if applicable).	Not applicable to this paper	

5. Describe what is already known about reliability and agreement and provide a rationale for the study (if applicable).	Not reliability, but explains the purpose of the paper which addresses intra-individual variability in UIC, as follows: *Several methods exist to correct for intra-individual variation in population survey data. One method is to collect multiple days of data on each survey participant and average the data for each participant. This has substantial logistical costs when conducting a national survey. Another method is to apply a correction factor to the distribution.[8, 9] This requires estimating the correction factor, for example by collecting multiple samples from a representative subset of the survey population.* *This second method has been applied many times to dietary intake data [10-12] but has been applied less frequently to biochemical data.[13-15]*	Page 5, last para and page 6, 1st para
METHODS		
6. Explain how the sample size was chosen. State the determined number of raters, subjects/objects, and replicate observations.	This was a convenient sample, no power calculation performed. Of the 110 volunteers, 84 were eligible for inclusion. N = 84 – this is similar to another study that assessed variability in Australian indigenous adolescents (Mackerras et al. [10].). Replicate observations (UIC) numbered three urine collections, taken one week apart.	Page 6
7. Describe the sampling method.	Volunteers were recruited from a random selection of aged care facilities (independent, assisted and low care living) in the Illawarra region, south of Sydney in Australia. Exclusion criteria described in detail.	Page 6
8. Describe the measurement/rating process (e.g. time interval between repeated measurements, availability of clinical information, blinding).	Replicate observations (UIC) numbered three urine collections, taken one week apart. Protocol for urine collection clearly described. Batch analysis of urinary iodine by the accredited laboratory of the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital (Sydney, NSW, Australia) described and coefficient of variation (CV) of the urinary iodine assay provided.	Page 7
9. State whether measurements/ratings were conducted independently.	Measurements were spot urine collections, same protocol followed for each collection and the same laboratory measured all samples. Accreditation of the laboratory for this analysis is described.	Page 7
10. Describe the statistical analysis.	*Descriptive analyses were performed using IBM SPSS(V19.0 IBM Corporation, Armonk NY). The 3-day repeated urinary iodine concentration estimates were used to determine a corrected UIC distribution after taking the natural logarithm of the iodine concentration. Repeated measures analysis of variance was performed to determine the between-person (sb) and total (sobs) standard deviations. A corrected UIC value was calculated for each person by adjusting the transformed value for each person, according to the following formula[15] using SAS (V9.2, SAS Cary, NC): Adjusted UIC = [(person's UIC – group mean) * (sb ÷ sobs)] + group mean. After exponentiation, the distribution of UICs was recalculated using the adjusted values and compared to the unadjusted mean values as well as the spot UIC collected on Day 1.*	Page 7, 2nd para
RESULTS		
11. State the actual number of raters and subjects/objects which were included and the number of replicate	See Table 2	Page 9

observations which were conducted		
12. Describe the sample characteristics of raters and subjects (e.g. training, experience)	Accreditation of the laboratory for this analysis is described.	Page 6, last para
13. Report estimates of reliability and agreement including measures of statistical uncertainty.	Estimates of reliability not appropriate to this study. Correct statistical results described as follows: *MUIC of the study population using the first spot urine collection indicated mild iodine deficiency (65.5 (IQR 42; 89)) µg/L). Correlations for transformed urinary iodine concentration values were: Days 1 and 2: r=0.48; p<0.01; Days 1 and 3: r=0.43; p<0.01; Days 2 and 3: r=0.41; p<0.01. The distribution of urinary iodine concentrations calculated by the different methods is shown in Table 2 and Figure 1. The s_b/s_{obs} was 0.83 when calculated using the three replicates and 0.79 when calculated using two replicates; i.e. the contraction in the distribution was slightly less with two replicates than three. Compared to the raw distribution, adjustment and averaging both reduced the spread of the distribution, especially at the upper end owing to the right skew in the data. Following adjustment using the three urine collections, the MUIC remained unchanged but the IQR was shifted upwards 65.2 (55; 94) while the upper end of the distribution (95th centile) changed from 176 to 136 µg/L. The percentage of participants with UIC ≥ 100 ug/L decreased from 21 % to 17 % following adjustment.*	Page 8, last para



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